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Effects of thiopental anesthesia on local rates of cerebral protein synthesis in rats

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Smith, C. Beebe, C. Eintrei, J. Kang, and Y. Sun. Effects of thiopental anesthesia on local rates of cerebral protein synthesis in rats. *Am. J. Physiol.* 274 (*Endocrinol. Metab.* 37): E852–E859, 1998.—We have examined the effects of a surgical level of thiopental anesthesia in adult male rats on local rates of cerebral protein synthesis with the quantitative autoradiographic L-[1-^{14}C]leucine method. The relative contribution of leucine derived from protein breakdown to the intracellular precursor amino acid pool for protein synthesis was found to be statistically significantly decreased in the anesthetized rats compared with controls. In the brain as a whole and in 30 of the 35 brain regions examined, rates of protein synthesis were decreased (1–11%) in the anesthetized rats. Decreases were statistically significant ($P \leq 0.05$) in the brain as a whole and in six of the regions, and they approached statistical significance in an additional 13 regions, indicating a tendency for a generalized but small effect.

leucine; brain; amino acid recycling; barbiturate

PROTEIN METABOLISM IN BRAIN is essential for both maintenance and growth of tissue, and it may underlie some of the special functions of the nervous system such as plasticity and memory formation. The effects of various pharmacological agents on brain protein synthesis are largely unknown. It is important to appreciate such effects because they may confound results of animal experiments or produce side effects in patients. Anesthetics, which are widely used in animal research in neuroscience, have been reported to cause large and generalized decreases in rates of protein synthesis in brain (4, 5, 8). These previous studies were beset with methodological problems. We have therefore reexamined the question of effects of barbiturate anesthesia on regional rates of protein synthesis in brain. In the present study, rates of protein synthesis were determined with a quantitative autoradiographic tracer method that measures the rate of incorporation of L-[1-^{14}C]leucine into protein (17). The method takes into account the dilution of the specific activity of the precursor amino acid pool in the tissue by unlabeled leucine derived from protein degradation. The study was designed to measure the effects of a surgical level of thiopental anesthesia on the rates of protein synthesis in localized regions of brain and in the brain as a whole, weighted for the masses of its component parts. Our results indicate that, in the brain as a whole and in most of the 35 brain regions examined, rates of protein synthesis are slightly decreased (5–11%) in thiopental-anesthetized rats.

METHODS

Chemicals. Chemicals and materials were obtained from the following sources: L-[1-^{14}C]leucine (sp act 55 mCi/mmol) from NEN, Wilmington, DE; L-[4,5-^{3}H]leucine (sp act 180 Ci/mmol) from American Radiolabeled Chemicals, St. Louis, MO; *Escherichia coli* tRNA from Sigma, St. Louis, MO; vanadyl ribonucleoside complex and redistilled nucleic acid-grade phenol from Bethesda Research Laboratories, Gaithersburg, MD; L-norleucine from Cyclochemicals, Division of Travenol Laboratories, Los Angeles, CA; and 5-sulfosalicylic acid from Fluka Chemie, Buchs, Switzerland.

Animals. All procedures were carried out in accordance with the National Institutes of Health Guidelines on the Care and Use of Animals and an animal study protocol approved by the National Institute of Mental Health Animal Care and Use Committee. Thirty-two normal male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 243–320 g were studied. Food and water were provided ad libitum. Rats were maintained under controlled conditions of normal humidity and temperature with standard alternating 12-h periods of light and darkness. Rats were prepared for experiments by insertion under light halothane anesthesia of polyethylene catheters into one femoral artery and both femoral veins. Catheters were tunneled under the skin to exit at the nape of the neck so that the rats could not gain access to the tubing. At least 3 h were allowed for recovery from the 20-min surgery before initiation of the experimental procedure. The experimental animals were reanesthetized with intravenous thiopental sodium (40–60 mg/kg) to a surgical level as indicated by the extinction of the corneal reflex (1, 16). Rats were maintained throughout the experimental procedure at this level by intravenous administration of thiopental sodium as required. Rats were anesthetized for 60 min before the initiation of measurements. Control animals were allowed to remain conscious and freely moving.

Physiological variables. Physiological variables were measured to evaluate each animal's physiological state. Mean arterial blood pressure, pH, P_{CO_2} and P_{O_2}, hematocrit, arterial plasma glucose concentration, and rectal temperature were measured as previously described (21).

Procedure to determine λ_{WB}, ψ_{WB}, *and* ψ_i. λ_i is the ratio of the integrated specific activity of leucine in the tissue precursor pool to that of the arterial plasma during at least 60 min after an intravenous pulse of labeled leucine

$$\lambda_i \approx \frac{\int_0^T [C_{pp}^*(t)/C_{pp}]\mathrm{d}t}{\int_0^T [C_p^*(t)/C_p]\mathrm{d}t} \qquad (1)$$

where C_{pp}^* and C_{pp} are the concentrations of the labeled and unlabeled leucine, respectively, in the precursor pool in *tissue i*, and C_p^* and C_p represent the concentrations in arterial plasma of labeled and unlabeled leucine, respectively. If the $C_p^*(t)/C_p$ is held constant for a long enough time (τ) for the tissue free and tRNA-bound leucine pools to reach a steady

state with the plasma, then

$$\lambda_i \approx \frac{C_{pp}^*(\tau)/C_{pp}}{C_p^*(\tau)/C_p} \qquad (2)$$

The time necessary to achieve this apparent steady state for leucine in the normal, conscious rat is between 30 and 60 min (17). The steady state is designated as apparent because it pertains to the free and tRNA-bound leucine pools only; a steady state for the [3H]leucine incorporated into peptide bonds in the protein is not even approached during the 60- or 90-min experiments.

Analogously, ψ_i is the steady-state ratio of the specific activity of leucine in the tissue acid-soluble amino acid pools to that of the arterial plasma

$$\psi_i \approx \frac{[C_E^* + C_M^*](\tau)/[C_E + C_M]}{C_p^*(\tau)/C_p} \qquad (3)$$

where C_E^* and C_E are the concentrations in the extracellular space of labeled and unlabeled leucine, respectively, and C_M^* and C_M are the concentrations in the intracellular metabolic pool of labeled and unlabeled leucine, respectively.

λ_{WB} and ψ_{WB} were evaluated in 12 rats by the method described by Smith et al. (17). Briefly, a constant arterial plasma specific activity for [3H]leucine was maintained for 60–90 min by means of a programmed infusion of [3H]leucine. The specific activities of [3H]leucine in arterial plasma and in the acid-soluble and tRNA-bound pools in brain were determined as described below.

Extraction and purification of aminoacyl-tRNA. The brain from each rat was homogenized by a motor-driven loose-fitting all-glass homogenizer in 10 ml of 0.25 M sucrose (0°C) containing 10 mM vanadyl ribonucleoside complex to inhibit RNase, 6 mg of uncharged *E. coli* tRNA as carrier, and L-norleucine (0.02 mM) added as an internal standard. The homogenates were centrifuged at 100,000 *g* for 1 h, and a pure aminoacyl-tRNA fraction was isolated from the supernatant fraction, as previously described (18). Briefly, the supernatant fraction was treated with TCA, the precipitated protein and nucleic acid were washed repeatedly to remove free amino acids, and the aminoacyl-tRNA was separated from protein by phenol extraction. The aminoacyl-tRNA was hydrolyzed at pH 10, and the specific activity of the previously tRNA-bound but now free amino acids was determined as described below.

Extraction of acid-soluble fraction in brain tissue. A 100-µl volume of the cytosol fraction, i.e., the supernatant solution derived from the 100,000 *g* centrifugation of the whole brain homogenates, was deproteinized by the addition of an equal volume of a solution of 8% (wt/vol) sulfosalicylic acid and stored at −70°C until assayed for leucine and [3H]leucine concentrations. At the time of these assays, the samples were thawed, mixed, and centrifuged for 30 min at 5,000 *g* at 4°C to remove the precipitated protein.

Dissection of brain regions. Brains were placed dorsal side up in a stainless steel rodent brain matrix (Activational System, Warren, MI), which was kept cold on crushed ice, and blades (Thomas Scientific, Swedesboro, NJ) were inserted through the slots in the matrix at right angles to the sagittal axis down to the level of cerebellum. The brains were sliced at intervals of 1 mm. Brain regions were dissected from these coronal slices (6). The remainder of the brain was placed on the chilled glass plate, and the cerebellum was completely detached from the brain stem and set aside. The hypoglossal nuclei were dissected bilaterally from the brain stem (24). Dissected brain regions were weighed, homogenized in 4% sulfosalicylic acid, which contained 2.5 µM norleucine as an

internal standard, and stored at −20°C until assayed for leucine and [3H]leucine concentrations. At the time of these assays, the samples were thawed, vortexed, and centrifuged for 30 min at 5,000 *g* at 4°C to remove the precipitated protein.

Assay of specific activity of [3H]leucine. Specific activities of [3H]leucine in deproteinized plasma, tissue acid-soluble fractions, and fractions derived from the deacylation of the aminoacyl-tRNA were assayed by post-column derivatization with *o*-phthaldehyde and fluorometric assay with a Beckman amino acid analyzer (model 7300, Beckman Instrument, Fullerton, CA). This system can measure 10–100 pmol of leucine with a 3% coefficient of variation. Fractions, after passage through the detector, were collected every minute and assayed for 3H with a Tri-Carb liquid scintillation analyzer (model 2250CA, Packard Instrument, Downers Grove, IL). Specific activity was calculated from total 3H in all fractions in the leucine peak and the total measured leucine content in the peak. The leucine concentration and the specific activity of [3H]leucine in the acid-soluble pool in the tissue were corrected for contamination by the leucine in the blood contained in the tissue. The equilibrium distribution of free leucine between erythrocytes and plasma was measured and found to be 0.67, and the hematocrit in brain was determined to be 30% (22).

Calculation of values of λ_{WB} and ψ_{WB}. Values of λ_{WB} were calculated from the ratio of the measured steady-state specific activity of the tRNA-bound leucine in the tissue to that of the acid-soluble leucine in arterial plasma (*Eq. 2*), and values of ψ_{WB} were calculated from the ratio of the measured steady-state leucine specific activity of the brain acid-soluble pool to that of the arterial plasma (*Eq. 3*). The time course of the specific activity in arterial plasma and the specific activities of acid-soluble and tRNA-bound leucine in whole brain at the end of the experimental period were determined as described above. The apparent steady-state free leucine specific activity in the arterial plasma was calculated as the mean of the specific activities determined from 40 min to the end of the experimental period. Whereas in some of the experiments the specific activity of leucine in the arterial plasma is not constant during the first 30 min, from 40 to 60 min (60-min experiments) and from 50 to 90 min (90-min experiments), values were within ±10% of the mean and showed no overall trend to increase or decrease over the entire interval. Leucine from the tRNA-bound amino acid fractions was uncontaminated by leucine derived from any blood in the brain tissue because of the procedure used to separate this fraction from the free amino acids in the tissue. The specific activity of [3H]leucine in the acid-soluble pools in the tissue was corrected for leucine in the residual blood contained in the tissue as described above.

Determination of local rates of protein synthesis. Local rates of protein synthesis were determined in 10 control and 10 thiopental-anesthetized rats. Rats were surgically prepared and catheterized, and their physiological states were monitored as described above. The procedure for the determination of the local rate of cerebral protein synthesis (lCPS_Leu) has been previously described (22). Briefly, the administration of an intravenous pulse of L-[1-14C]leucine (100 µCi/kg) initiated the procedure. Timed arterial blood samples were collected during the following 60 min for determination of the time courses of plasma concentrations of leucine and [14C]leucine. At the end of the 60-min experimental period, rats were killed by an intravenous injection of pentobarbital sodium, and the brains were rapidly removed and frozen in isopentane cooled to −40°C with dry ice. Brain sections were prepared and autoradiographed along with calibrated

[^{14}C]methylmethacrylate standards as previously described (22). The rates of leucine incorporation into protein in individual brain regions and the average for the brain as a whole, weighted for the relative masses of its component parts, were determined by analysis of the autoradiograms with a computerized image-processing system (MCID Imaging Research, St. Catharines, ON, Canada) with a pixel size of 28 μm. Regions were located according to the rat brain atlas of Paxinos and Watson (13). The concentration of ^{14}C in each region of interest in the autoradiograms was determined from the optical density vs. ^{14}C concentration curve for the calibrated plastic standards. Local rates of protein synthesis were calculated by means of the operational equation of the method (17) with the condition-specific values of λ_i

$$R_i = \frac{P_i^*(T)}{\lambda_i \left[\int_0^T \frac{C_p^*(t)}{C_p} \, dt \right]} \qquad (4)$$

where R_i is the rate of leucine incorporation into protein in *tissue i*; $P_i^*(T)$ is the concentration of ^{14}C fixed in the *tissue i* at any time, T, after introduction of the tracer into the circulation; λ_i is equal to the fraction of leucine in the precursor pool for protein synthesis in the *tissue i* that is derived from plasma; and t is variable time. Rates of protein synthesis measured with the [^{14}C]leucine method are rates of incorporation of leucine into all tissue protein, which is comprised of a mixture of many individual proteins. The rate of leucine incorporation into each individual protein is weighted according not only to its individual rate of turnover but also to the fraction of total tissue protein that it comprises.

Statistics. Values of λ_{WB}, ψ_{WB}, λ_i, and ψ_i and weighted average rates of protein synthesis and lCPS$_{Leu}$ in 35 brain structures determined in thiopental-anesthetized rats were compared with those of controls by means of Student's t-tests. Results from one control animal were not included in the series because its endogenous arterial plasma leucine concentration decreased by 32% during the course of the 60-min experiment.

RESULTS

Physiological status. Sixty minutes of surgical anesthesia with thiopental produced statistically significant changes in some of the physiological variables that were measured (Table 1). Arterial blood P$_{O_2}$ was decreased 16%, P$_{CO_2}$ was increased 28%, and pH was decreased 1% in the anesthetized rats compared with

Table 1. *Physiological variables in control and thiopental-anesthetized rats*

	Control	Thiopental Anesthetized
Body weight, g	287 ± 6	282 ± 7
Rectal temperature, °C	37.9 ± 0.1	38.0 ± 0.1
Mean arterial blood pressure, mmHg	107 ± 3	$97 \pm 2^*$
Arterial plasma glucose concn, mg/100 ml	178 ± 5	$149 \pm 3^*$
Arterial hematocrit	0.45 ± 0.01	0.47 ± 0.01
Arterial blood P$_{CO_2}$, mmHg	41 ± 1	$53 \pm 2\dagger$
Arterial blood P$_{O_2}$, mmHg	86 ± 1	$72 \pm 3^*$
Arterial blood pH	7.43 ± 0.01	$7.36 \pm 0.01\dagger$
Arterial plasma leucine concn, nmol/ml	150 ± 12	167 ± 5

Values are means $\pm$ SE; $n = 10$ rats for each group. *Statistically significantly different from controls, $P \leq 0.01$ (Student's t-test). †Statistically significantly different from controls, $P \leq 0.00001$ (Student's t-test).

Table 2. *Effects of thiopental anesthesia on leucine concentrations in plasma and brain amino acid pools*

	Normal Conscious Rats ($n = 9$)	Thiopental-Anesthetized Rats ($n = 12$)
Arterial plasma leucine concn, nmol/ml	154 ± 9	$188 \pm 6^*$
Brain acid-soluble leucine pool, nmol/g	55 ± 4	$70 \pm 3^*$
Brain acid-soluble to plasma distribution ratio for leucine	0.36 ± 0.03	0.37 ± 0.02
Brain tRNA-bound leucine pool, nmol/g	0.096 ± 0.006	$0.113 \pm 0.003^*$

Values are means $\pm$ SE. Measurements in control rats were made at the time that these studies of thiopental anesthesia were being carried out; these results have been reported previously (21). Measurements were made in thiopental-anesthetized rats in which λ_{WB} was evaluated. *Statistically significantly different from controls, $P \leq 0.01$ (Student's t-test).

the controls. Arterial plasma glucose concentration was 17% lower and mean arterial blood pressure was 9% lower in the anesthetized rats. Rectal temperature and hematocrit were unchanged. The behavior of the control animals was normal; they were alert but calm. The anesthetized animals were unresponsive to stimulation throughout the experimental period.

Effects of thiopental anesthesia on leucine concentrations in brain amino acid pools. Leucine concentrations in both the total acid-soluble and tRNA-bound amino acid pools extracted from whole brain of the anesthestized rats were statistically significantly higher than those of controls (Table 2). Tissue acid-soluble to plasma distribution ratios, however, were similar in the two groups, indicating that the higher leucine concentration in the acid-soluble pool merely reflects a higher arterial plasma leucine concentration in the anesthetized animals.

Effects of thiopental anesthesia on values of λ_i and ψ_i. In 12 thiopental-anesthetized rats, a constant arterial plasma [^{3}H]leucine concentration was maintained for a duration of 60 ($n = 4$) or 90 ($n = 8$) min, and the ratios of [^{3}H]leucine specific activities in tissue pools to that of arterial plasma were determined at the end of the infusion. The ratio in the tRNA-bound pool was similar at both time points, 0.62 ± 0.01 at 60 min and 0.64 ± 0.01 (mean $\pm$ SE) at 90 min, indicating that a steady state had been achieved by 60 min. In the acid-soluble pool, the ratio increased between 60 and 90 min from 0.52 ± 0.01 to 0.59 ± 0.01 (mean $\pm$ SE); these values are statistically significantly different from one another ($P = 0.0006$, Student's t-test), indicating that the acid-soluble pool turns over more slowly and has a longer half-life than the tRNA-bound pool. We have assumed that a steady state was achieved in the acid-soluble pool by 90 min. In conscious rats, the tRNA-bound and acid-soluble pools reach apparent steady states after 30 and 60 min, respectively, of a constant arterial [^{3}H]leucine concentration. It is not unreasonable to allow 30 min beyond the attainment of a steady state in the tRNA-bound pool for the acid-soluble pool to reach a steady state with the plasma in

the thiopental-anesthetized rats. We have therefore used the ratios at 90 min of 0.64 and 0.59 as the values for λ_{WB} and ψ_{WB}, respectively. Both of these values are statistically significantly ($P < 0.001$) higher than the values of λ_{WB} and ψ_{WB} in conscious adult rats (22).

We have previously shown that in conscious adult rats, values of λ_{WB} correlate closely with the values of ψ_{WB} (22) and that the relationship could be fitted quite well by a linear equation ($\lambda_{WB} = 0.29 + 0.60\psi_{WB}$). The best-fitting linear relationship between λ_{WB} and ψ_{WB} is unchanged by the addition of the results of our 90-min experiments in the thiopental-anesthetized rats to the original series of controls (Fig. 1). The correlation between λ_{WB} and ψ_{WB} for the combined group remains statistically significant ($r_{xy} = 0.91$, $P < 10^{-6}$), and agreement between the measured values of λ_{WB} and those estimated by the linear equation was excellent. No statistically significant improvement in fit was found with polynomial regressions of higher degrees.

Regional values of λ_i were estimated from the linear relationship between ψ_{WB} and λ_{WB} and experimentally determined values of ψ_i. Four thiopental-anesthetized rats already in a steady state for unlabeled leucine were given programmed intravenous infusions of [^{3}H]leucine that maintained the specific activity of [^{3}H]leucine in arterial plasma relatively constant long enough (90 min) for the acid-soluble leucine pool in brain tissue to reach an approximate steady state with respect to plasma. In all four experiments, the specific activities of the acid-soluble leucine pool in all 13 regions examined were below those of arterial plasma. The values of ψ_i ranged from 0.45 in substantia nigra to 0.60 in the hypoglossal nucleus (Fig. 2). The data were statistically analyzed for homogeneous subgroups of these values by repeated-measures ANOVA. One subgroup was found within which there were no significant differences in the values of ψ_i; this group was composed of all regions of gray matter except substantia nigra, globus pallidus, and hypoglossal nucleus. The mean $\pm$ SE value in this group was 0.54 ± 0.007. The values in the other regions were outside the range of this homogeneous subgroup. Values for λ_i (Table 3) were calculated for each region based on the linear equation (Fig. 1) fitted from the determined values of λ_{WB} and ψ_{WB}.

Fig. 2. Experimentally determined regional values of ψ_i in thiopental-anesthetized rats. Data are mean $\pm$ SE (bars) values from determinations in 4 rats in all regions except globus pallidus and internal capsule, in which determinations were made in 3 rats. Regional values were tested for homogeneity by means of repeated-measures ANOVA. One subgroup of gray matter regions (see RESULTS) was found to be homogeneous. Abbreviations are as follows: SN, substantia nigra; GP, globus pallidus; V CX, visual cortex; A CX, auditory cortex; Thal, thalamus; MG, medial geniculate; IColl, inferior colliculus; F CX, frontal cortex; Acb, nucleus accumbens; CP, caudate putamen; IC, internal capsule; CC, corpus callosum; XII, hypoglossal nucleus.

Examination of these data for homogeneous subgroups by repeated-measures ANOVA showed the same subgroup of gray matter as was found with the values of ψ_i.

Effects of thiopental anesthesia on lCPS$_{Leu}$. Autoradiograms from anesthetized rats were not apparently different from those of controls (Fig. 3). Rates of leucine incorporation into protein were determined in the brain as a whole and in 35 brain regions (Table 4). In the brain as a whole, lCPS$_{Leu}$ was decreased by 9% ($P = 0.026$) in the anesthetized rats. In 30 of the 35 regions examined, rates of protein synthesis were lower in the anesthetized rats than in the controls. Decreases in lCPS$_{Leu}$ were statistically significant ($P \le 0.05$) in six of
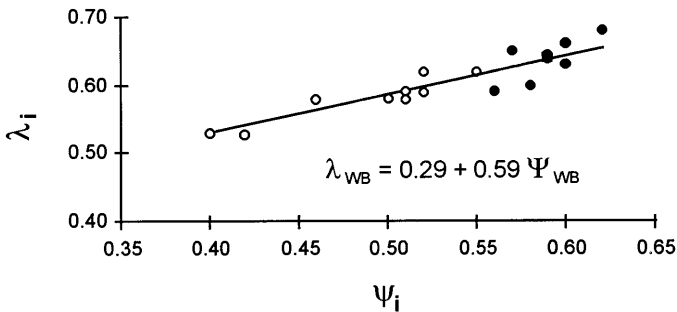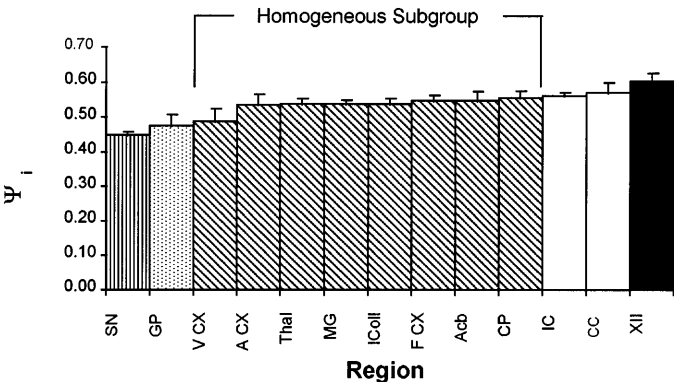

Fig. 1. Relationship between experimentally determined values of ratios ψ_{WB} and λ_{WB}. Each point represents determinations of both values in a single rat. Ratios were determined in 9 conscious rats (○) and 8 thiopental-anesthetized rats (●). Best-fitting straight line and equation for line are illustrated. Correlation coefficient for fit was 0.91 ($P < 10^{-6}$).

$$\lambda_{WB} = 0.29 + 0.59\,\Psi_{WB}$$

Table 3. *Regional values of λ_i in thiopental-anesthetized rats*

Brain Region	λ_i
Cortex	
Frontal cortex, areas 1–3	0.61 ± 0.01
Auditory cortex	0.61 ± 0.02
Visual vortex	0.58 ± 0.02
Subcortical structures	
Nucleus accumbens	0.61 ± 0.02
Caudate putamen	0.62 ± 0.01
Globus pallidus	0.57 ± 0.02
Thalamus	0.61 ± 0.01
Medial geniculate	0.61 ± 0.01
Substantia nigra	0.55 ± 0.01
Inferior colliculus	0.61 ± 0.01
White matter	
Corpus callosum	0.63 ± 0.02
Internal capsule	0.62 ± 0.01
Brain stem	
Hypoglossal nucleus	0.65 ± 0.01
Whole brain	0.64 ± 0.0001

Values are means $\pm$ SE from 4 rats except in globus pallidus and internal capsule, in which determinations were made in 3 rats. Values were calculated from regional values of ψ_i (Fig. 2). Linear equation shown in Fig. 1 was used for these calculations.

Control   **Thiopental**

cc
CP
PC
ox

PVN
BLA

SC
Te3
MG

OC1
IC

Pr5

5 mm

Fig. 3. Representative [^{14}C]leucine autoradiograms from control (*left*) and thiopental-anesthetized (*right*) rats. Abbreviations are as follows: cc, corpus callosum; CP, caudate putamen; PC, piriform cortex; ox, optic chiasm; PVN, paraventricular hypothalamic nucleus; BLA, basolateral amygdaloid nucleus; SC, superior colliculus; Te3, auditory cortex; MG, medial geniculate nucleus; OC1, visual cortex; IC, inferior colliculus; Pr5, trigeminal nucleus.
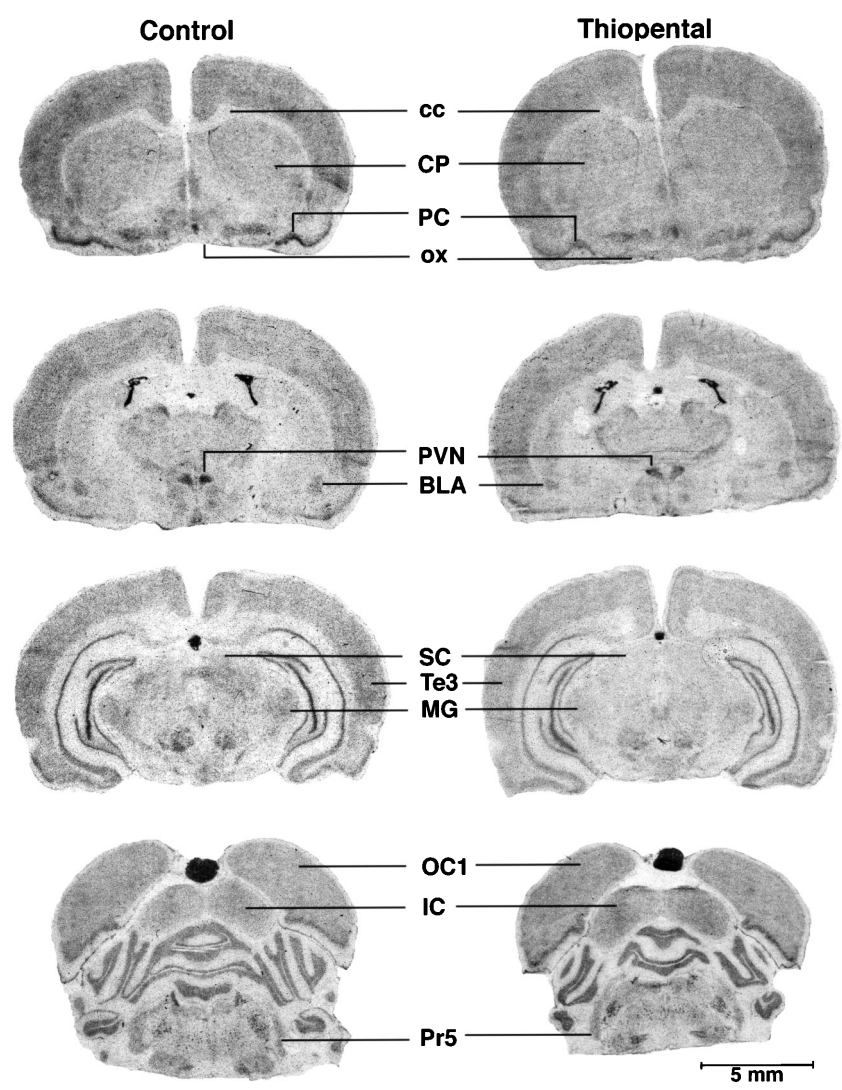
the regions; the magnitude of these decreases was 9–11% (Table 4).

DISCUSSION

The results of the present studies show that surgical barbiturate anesthesia decreases rates of protein synthesis in the brain. The effects are fairly small in magnitude, i.e., ~10%, and appear to be generalized. Rates of protein synthesis reported in these studies are the actual rates of leucine incorporation into protein because the equation (*Eq. 4*) used to calculate lCPS$_{Leu}$ includes a factor, λ_i, which corrects the integrated specific activity of the labeled leucine measured in arterial plasma for the contribution of unlabeled leucine derived from protein degradation in the tissue. The values of λ_i used in the calculations were condition specific (conscious vs. thiopental anesthetized) as well as region specific. Values for λ_{WB} were determined experimentally in control and thiopental-anesthetized rats, and values for λ_i were estimated from measured values of ψ_i for the specific regions analyzed under both conditions. Without correction for recycling, rates of protein synthesis determined with a radiolabeled amino acid tracer may be underestimated.

Methodological considerations. Results of previous studies with [^{35}S]methionine as the tracer have suggested that large and generalized decreases in rates of protein synthesis in brain occur with various anesthetic agents (5, 8). In neither of these studies, however, was dilution of the precursor pool by unlabeled methionine derived from protein degradation measured. In addition, there are problems with side reactions and significant levels of nonspecific labeled metabolic products of [^{35}S]methionine, which appear in both the brain and the blood. In both of these studies, concerns were raised that the effects on protein synthesis may actually have been due to reductions in body temperature that accompany anesthesia. In our studies, all of these potential problems have been circumvented.

In our calculations of lCPS$_{Leu}$, we included a factor λ_i in the equation to correct for the dilution of the precursor pool by unlabeled leucine from protein breakdown. We have determined λ_{WB} and estimated regional values for λ_i under both control and anesthetized conditions. Values of λ_i are functions of steady-state measurements of ψ_i. In control animals, we verified that a steady state was reached at the time of the measurements, but in the anesthetized rats, we have

Table 4. *Effects of thiopental anesthesia on whole brain and regional rates of leucine incorporation into protein (nmol·g^{-1}·min^{-1})*

Brain Region	Controls ($n=9$)	Anesthetized ($n=10$)	P Value	%Change
Thalamus	6.7 ± 0.2	5.9 ± 0.2	0.007	−11
Auditory cortex	6.5 ± 0.2	5.9 ± 0.2	0.012	−10
Septal nucleus, medial*	5.9 ± 0.2	5.3 ± 0.1	0.016	−11
Whole brain weighted average	5.4 ± 0.2	4.9 ± 0.1	0.026	−9
Corpus callosum	3.8 ± 0.2	3.4 ± 0.1	0.028	−11
Frontal cortex, areas 1–3	5.6 ± 0.2	5.0 ± 0.1	0.038	−10
Anterior cingulate cortex, area 3*	6.4 ± 0.3	5.8 ± 0.1	0.042	−10
Superior colliculus, superficial layer*	5.5 ± 0.2	5.0 ± 0.2	0.055	−9
Nucleus accumbens, shell*	5.0 ± 0.1	4.7 ± 0.1	0.060	−7
Medial geniculate	5.9 ± 0.2	5.5 ± 0.2	0.067	−8
Internal capsule	3.6 ± 0.1	3.3 ± 0.1	0.073	−8
Nucleus accumbens	5.0 ± 0.3	4.4 ± 0.1	0.073	−11
Ventral medial hypo-thalamus*	6.5 ± 0.2	5.8 ± 0.1	0.076	−6
Basolateral amygda-loid nucleus*	6.2 ± 0.2	5.8 ± 0.2	0.087	−6
Trigeminal tract*	2.8 ± 0.1	2.5 ± 0.1	0.090	−10
Zona incerta*	5.8 ± 0.2	5.3 ± 0.2	0.091	−8
Nucleus accumbens, core*	4.2 ± 0.1	3.8 ± 0.1	0.094	−7
Medial forebrain bundle*	6.6 ± 0.2	6.1 ± 0.1	0.105	−7
Frontal cortex, area 1	5.9 ± 0.2	5.5 ± 0.1	0.107	−7
Optic chiasm*	2.9 ± 0.1	2.6 ± 0.1	0.121	−9
Cerebellum, molecular layer*	3.9 ± 0.1	3.6 ± 0.1	0.166	−7
Globus pallidus	4.3 ± 0.2	4.0 ± 0.1	0.172	−7
Piriform cortex*	9.1 ± 0.4	8.5 ± 0.2	0.209	−6
Inferior colliculus	6.8 ± 0.2	6.5 ± 0.2	0.225	−5
Visual cortex	6.3 ± 0.2	6.0 ± 0.2	0.255	−5
Principal sensory tri-geminal nucleus*	6.6 ± 0.2	6.4 ± 0.2	0.273	−4
Lateral amygdaloid nucleus*	5.1 ± 0.2	4.9 ± 0.2	0.352	−4
Caudate putamen	4.7 ± 0.2	4.6 ± 0.1	0.379	−4
Dentate gyrus, poly-morph layer*	9.0 ± 0.3	9.2 ± 0.2	0.515	3
Substantia nigra	4.5 ± 0.2	4.4 ± 0.2	0.525	−3
Hypoglossal nucleus	7.8 ± 0.3	7.9 ± 0.2	0.675	2
Olfactory bulb, mitral cell layer*	6.8 ± 0.3	6.7 ± 0.2	0.761	−2
Cerebellum, Purkinje cells*	8.4 ± 0.3	8.5 ± 0.3	0.799	1
Tenia tecta, dorsal*	7.8 ± 0.3	7.7 ± 0.2	0.814	−1
Septal nucleus, lateral*	4.5 ± 0.1	4.5 ± 0.1	0.905	−0
Hippocampus, CA1 pyramidal cells*	7.4 ± 0.2	7.4 ± 0.1	0.959	−0

Values are means $\pm$ SE of measurements obtained from no. of animals (*n*) except in olfactory bulb, in which measurement were made in 5 controls and 7 anesthetized rats, and in medial forebrain bundle, in which measurements were made in 8 controls. Regional rates of protein synthesis were calculated with the values of λ_i given in Table 3. *P* values were determined with Student's *t*-tests. *Values of λ_i were estimated as follows. In anesthetized rats, mean value of λ_i (0.61 ± 0.004, mean $\pm$ SE) for homogeneous group of gray matter regions was used for all gray matter regions. In white matter regions, mean value (0.62 ± 0.003) for internal capsule and genu of the corpus callosum was used. In control animals, values for λ_i were similarly estimated. In most regions of gray matter, we used the value of λ_i (0.56 ± 0.002, mean $\pm$ SE for 6 regions) calculated from mean value of ψ_i for group II (22). In white matter regions, we used value of λ_i (0.54 ± 0.003, mean $\pm$ SE for 6 regions) calculated from mean value of ψ_i for group I (22). In frontal cortex, we used the specific value for that region of 0.57 (22).

assumed that a steady state was reached after 90 min of a programmed infusion of labeled leucine. If, in some brain regions, a steady state was not achieved at 90 min, we may have underestimated ψ_i and consequently the true value of λ_i. An underestimation of λ_i would result in an overestimation of lCPS$_{Leu}$. The effect on the final results would be an underestimation of the magnitude of the reduction in lCPS$_{Leu}$ by thiopental.

Extraneous labeled metabolites were avoided by the use of carboxyl-labeled leucine because the label is transiently converted to α-[1-^{14}C]ketoisocaproic acid and then to ^{14}CO$_2$. Labeled CO$_2$ in the plasma is removed during the deproteinization step, and we have measured the time course of the appearance of α-[1-^{14}C]ketoisocaproic acid in the plasma after a pulse injection of [1-^{14}C]leucine in both controls and thiopental-anesthetized rats. In both cases, the integrated activity of α-[^{14}C]ketoisocaproic acid is only 5% of the total integrated activity of the acid-soluble pool of ^{14}C in the plasma. Finally, in our experiments, rectal temperature was maintained at 38°C with a heat lamp thermostatically controlled by a thermistor temperature-sensing probe.

Tissue energy use and protein synthesis rates. Studies in tissues other than brain have also shown effects of anesthesia on leucine and phenylalanine metabolism. Studies of whole body leucine metabolism in dogs (7) have indicated that leucine incorporation into protein decreases during halothane anesthesia. Others have reported that, in both perfused lung and perfused heart, pentobarbital (342 g/ml) and halothane (1–4%) inhibit phenylalanine incorporation into protein (14). These effects of pentobarbital on heart were accompanied by 50% decreases in creatine phosphate; levels of ATP were unchanged. In barbiturate-anesthetized brain, where energy use is reduced (9, 19, 20, 23), high-energy phosphate levels remain normal or are elevated (10, 12), but rates of protein synthesis appear to be slightly reduced. We have seen changes in rates of protein synthesis under conditions in which there may be a depletion of ATP, such as during focal motor seizures (3). During seizures, rates of glucose utilization were increased severalfold in the focus and in structures in direct communication with the focus, but in these same areas, rates of protein synthesis were reduced.

Effects of thiopental anesthesia on values of λ_i. Values of λ_i calculated for specific brain regions were homogeneous in most gray matter regions excluding globus pallidus, substantia nigra, and hypoglossal nucleus. Mean values for the two white matter regions examined were very similar, but there are not adequate data to confirm that values in all white matter regions are the same. Values for λ_i across regions in control (22) and thiopental-anesthetized rats were compared by repeated-measures ANOVA. The two groups are statistically significantly different from one another. Values for λ_i are clearly higher in all regions in the thiopental-anesthetized rats compared with controls. Whereas values for λ_i were lowest in the globus pallidus and substantia nigra and highest in the hypoglossal nucleus in both groups, values for λ_i in white matter were

relatively lower in control animals compared with the anesthetized rats. In the thiopental-anesthetized rats, values in white matter were among the highest measured, suggesting that changes in rates of either protein degradation or delivery of amino acids from the plasma are greater in white matter than in gray matter regions, which may reflect the high solubility of thiopental in lipids with a consequent higher tissue concentration in white matter. Values of ψ_{WB} determined in thiopental-anesthetized rats were also higher than in controls, but by 20%, indicating that the total tissue acid-soluble leucine pool is more affected by thiopental than the tRNA-bound pool.

Effects of thiopental anesthesia on leucine transport and protein degradation. The results of several studies (2, 15) suggest that barbiturates may increase transport of neutral amino acids into brain. If so, then the balance between the contributions to the precursor amino acid pool from the plasma and protein breakdown could be altered, which would in turn have an effect on the value of λ as follows

$$\lambda = \frac{\text{flux}_{\text{plasma}}}{\text{flux}_{\text{plasma}} + \text{flux}_{\text{protein breakdown}}} \qquad (5)$$

By rearranging and substituting lCPS_{Leu} for the sum of the fluxes from plasma and protein breakdown

$$\text{flux}_{\text{plasma}} = \lambda \cdot \text{lCPS}_{\text{Leu}} \qquad (6)$$

and

$$\text{flux}_{\text{protein breakdown}} = \text{lCPS}_{\text{Leu}} - \text{flux}_{\text{plasma}} \qquad (7)$$

The flux of leucine from the plasma into the precursor amino acid pool for protein synthesis calculated from measured rates of protein synthesis and values of λ_{WB} is 3.1 nmol·g^{-1}·min^{-1} in both controls and anesthetized rats, indicating no effect on net transport across the blood-brain barrier. In contrast, the calculated flux from protein breakdown is decreased by 22% in anesthetized rats from 2.3 in controls to 1.8 nmol·g^{-1}·min^{-1}, indicating a decreased rate of protein degradation in the brain as a whole under these conditions. In the 13 individual brain regions in which ψ_i was determined and λ_i was calculated, results were similar to those in whole brain; i.e., calculated fluxes of leucine into tissue precursor pools from plasma were similar under both conditions, whereas fluxes from protein degradation were consistently lower in the thiopental-anesthetized rats. The effect of greatest magnitude (30% decrease) was found in corpus callosum.

Effects of thiopental anesthesia on lCPS$_{Leu}$. Thiopental anesthesia reduced lCPS$_{\text{Leu}}$ in the brain as a whole by 9%. In an effort to determine whether this was a general or regionally specific effect, we analyzed lCPS$_{\text{Leu}}$ in 35 brain regions. By standard statistical analysis (Student's t-tests), six of the regions had statistically significant decreases in lCPS$_{\text{Leu}}$ ($P \leq 0.05$). If we apply the very conservative correction for multiple comparison statistics by the method of Bonferroni, none of the regions is statistically significantly affected by thiopental, but the preponderance of P values (~50%) at or close to statistical significance ($P \leq 0.1$) supports a more generalized effect.

Perspectives. These investigations show that surgical thiopental anesthesia in rats results in widespread but small decreases in rates of cerebral protein synthesis. The results of our analyses of fluxes from protein degradation suggest that rates of protein degradation may also be decreased throughout the brain. The biological or clinical significance of these relatively small effects of thiopental anesthesia is uncertain. A small inhibition in protein synthesis in the nervous system counterbalanced by a decrease in breakdown indicates that turnover is slowed but that there should be no net loss of protein. Perhaps under anesthesia, when the brain is relatively inactive, a reduced protein turnover rate would not be harmful to the tissue. The mechanism by which thiopental effects this reduction is probably not related to activity per se, since we have found that protein synthesis rates in adult monkeys are positively correlated with the time in the inactive state of deep sleep (11). Effects might be larger after more prolonged anesthesia and/or at deeper levels of anesthesia. We cannot ascertain from these studies whether the cells affected are neurons or glia or both. Interestingly, results for the regions that are rich in neuronal cell bodies suggest that there is very little effect on neurons. In contrast, both protein synthesis rates and fluxes from protein degradation in areas rich in neuropil and white matter appear to be consistently reduced in the thiopental-treated rats. This apparent predilection for effects in white matter and neuropil may reflect the higher solubility of thiopental in these more lipid-rich areas of brain.

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